# Development of Rapid Response Method for Organophosphorus Pesticide Exposure by Analyzing Non-Specific Urinary Metabolites

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## INTRODUCTION

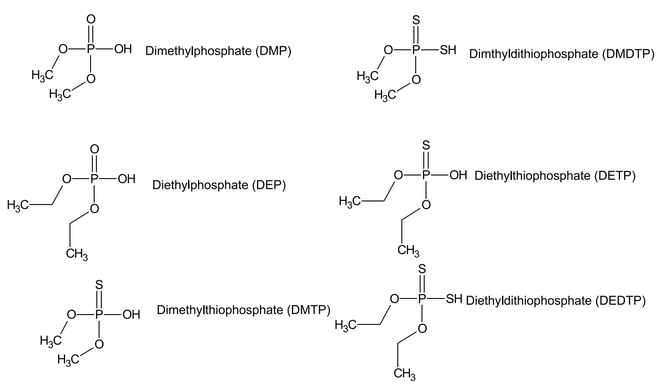
The possibility of organophosphorus (OP) pesticides being employed as weapons of chemical terrorism is present because they can be toxic to humans and are accessible and inexpensive. Post September 11, 2001, the Department of Homeland Security has called upon state laboratories to help the CDC in response to chemical terrorist activities. Therefore, implementing analytical methods to assess OP pesticide exposure is necessary within state laboratories.

Currently, CDC assesses total OP pesticide exposure by analysis of non-specific dialkylphosphates (DAP) metabolites (6) in urine. This method involves lyophilization, chemical derivitization and analysis with gas chromatography-tandem mass spectrometry. It is characterized by low detection levels and high precision and accuracy. However, it is long (40 hours per 50 samples) and involves expensive equipment and chemicals which may be difficult to integrate into state laboratories. Ideally, the method would utilize standards and instrumentation that are cost effective and also be rapid in case of terrorism activities.

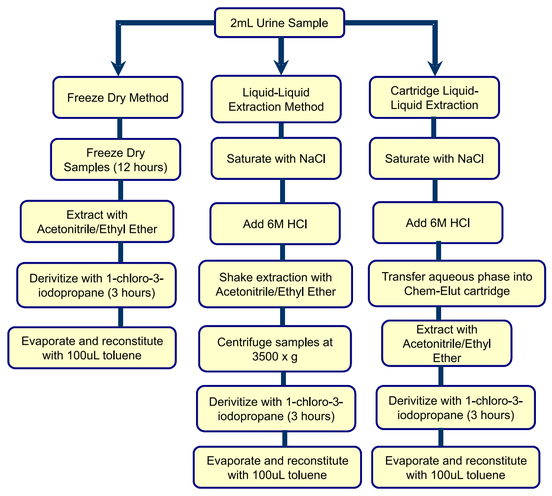
We have investigated three analytical methods determining concentrations of DAPs in urine to establish which method is superior in terms of detection limits and response time. One method utilizing sorbent-immobilized cartridges reduces sample preparation time by 50% although limits of detection are higher. However, the method would be suitable for chemical terrorism response to organophosphate exposure, because predictably, higher concentrations would be observed.

The developed method is practical for state laboratories. Less expensive equipment and chemicals are required and it is rapid for chemical terrorism situations. This analysis coincides with a national public health need to analyze OP pesticides as possible weapons of chemical terrorism in response to our changing world that increasingly emphasizes chemical terrorism defense and response.

## DIALKYLPHOSPHATE STRUCTURES




## SAMPLE PREPARATION METHODS




## INSTRUMENTAL CONDITIONS

### CHROMATOGRAPHIC CONDITIONS

**Gas Chromatograph (MS/MS):** ThermoFinnigan Trace GC + with CTC A200S autosampler
**Gas Chromatograph (MSD):** HP6890 + HP7683 Injector autosampler

**Column:** 30 m J&W DB-5MS (0.25 µm film thickness, 0.25 mm I.D., capillary Column, 5% phenyl]-methyl polysiloxane )

**Injection:** 1 µl injected using splitless injection.

**GC Program:** The injector and transfer line temperatures were 250 °C. The column temperature was initially 80 °C for 2 min and was then heated to 235°C at 17°C/min and then to 270 °C at 35 °C/min. The final temperature of 270 °C was held for 5 min.

### MASS SPECTROMETRIC CONDITIONS

**Mass Spectrometer (MS/MS):** Triple quadrupole mass spectrometer (FinniganTSQ-7000).

The analytes were analyzed using selected reaction monitoring (SRM).

The source temperature was 150 °C, electron energy was 200 eV. Methane was used as a reagent gas with a pressure of 1500 mT and argon as a collision induced dissociation gas with a pressure of 2 mT.

Mass Spectrometer (MSD): HP5973 MSD

The analytes were analyzed using single ion monitoring (SIM).

The source temperature was 250°C, quadrupole temperature was 150°C with electron energy of 156eV and electron multiplier at 1700V. Methane was used as reagent gas with gas flow set at 20%.

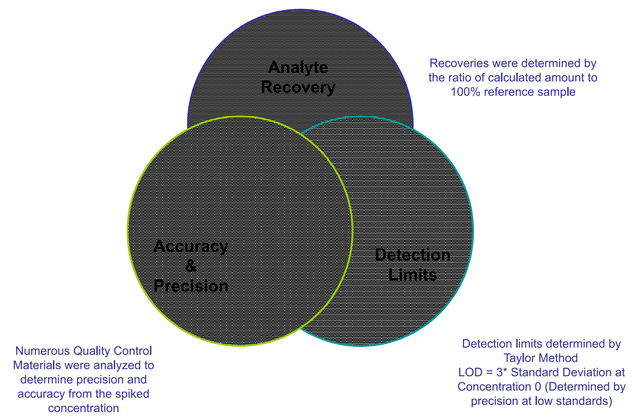
**SINGLE ION MONITORING METHOD SPECIFICATIONS**

| ANALYTE | ION MASS |
|---|---|
| DMP | 203 |
| ᴸDMP | 209 |
| DEP | 231 |
| ᴸDEP | 241 |
| DMTP | 219 |
| ᴸDMTP | 225 |
| DMDTP | 235 |
| ᴸDMDTP | 241 |
| DETP | 247 |
| ᴸDETP | 257 |
| DEDTP | 263 |
| ᴸDEDTP | 267 |
| DBP | 287 |
| ᴸ Label Internal Standard | |

**SELECTED REACTION MONITORING METHOD SPECIFICATIONS**

| ANALYTE | COLLISION ENERGY (eV) | PRECURSOR ION MASS | PRODUCT ION MASS |
|---|---|---|---|
| DMP | -12.0 | 203 | 127 |
| ᴸDMP | -12.0 | 209 | 133 |
| DEP | -13.0 | 231 | 127 |
| ᴸDEP | -13.0 | 241 | 132 |
| DMTP | -13.0 | 219 | 143 |
| ᴸDMTP | -13.0 | 225 | 149 |
| DMDTP | -10.0 | 235 | 125 |
| ᴸDMDTP | -10.0 | 241 | 131 |
| DETP | -12.0 | 247 | 191 |
| ᴸDETP | -12.0 | 257 | 193 |
| DEDTP | -12.0 | 263 | 153 |
| ᴸDEDTP | -12.0 | 267 | 157 |
| DBP | -10.0 | 287 | 175 |

## VALIDATION PARAMETERS

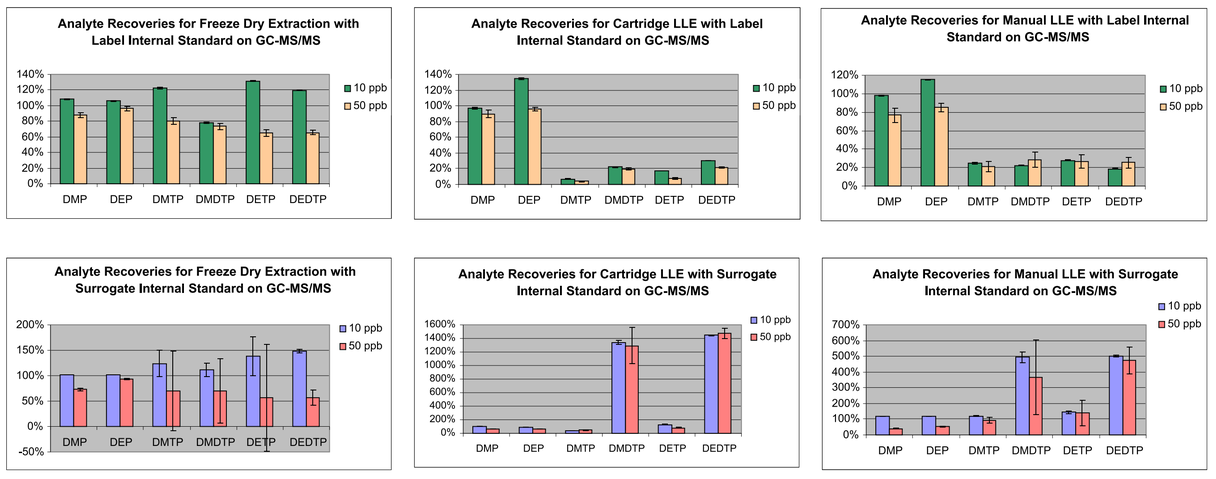



Recoveries were determined by the ratio of calculated amount to 100% reference sample

Numerous Quality Control Materials were analyzed to determine precision and accuracy from the spiked concentration

Detection limits determined by Taylor Method
LOD = 3* Standard Deviation at Concentration 0 (Determined by precision at low standards)

## RECOVERIES COMPARING LABEL INTERNAL STANDARD VS SURROGATE STANDARD FOR 3 CLEAN -UP PROCEDURES IN GC-MS/MS




## DETECTION LIMITS FOR ALL THREE CLEAN-UPS ON GC-MS/MS (ppb)

| LOD-TSQ | DMP | DEP | DMTP | DMDTP | DETP | DEDTP |
|---|---|---|---|---|---|---|
| Freeze Dry ISTD | 0.34 | 2.85 | 0.96 | 0.18 | 0.07 | 0.37 |
| LLE Cartridge ISTD | 0.86 | 2.97 | 0.22 | 0.02 | 0.02 | 0.01 |
| LLE hand ISTD | 0.01 | 1.72 | 0.51 | 0.25 | 0.05 | 0.15 |
| Freeze Dry DBP | 0.03 | 0.20 | 0.07 | 0.23 | 0.29 | 0.26 |
| LLE Cartridge DBP | 1.37 | 2.00 | 0.83 | 0.53 | 0.40 | 0.31 |
| LLE hand DBP | 4.08 | 0.90 | 1.23 | 1.04 | 0.12 | 0.02 |

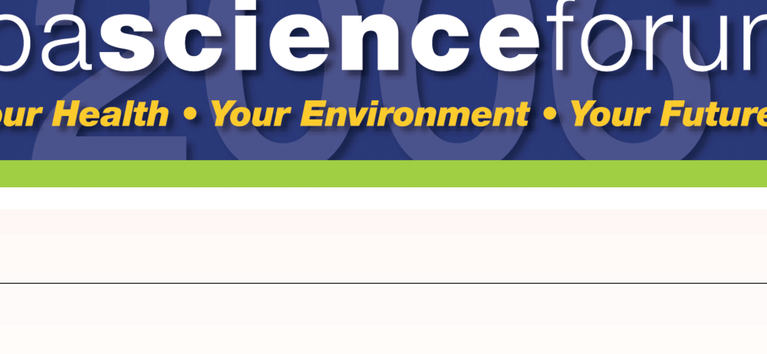
| LOD-MSD | DMP | DEP | DMTP | DMDTP | DETP | DEDTP |
|---|---|---|---|---|---|---|
| Freeze Dry ISTD | 13.1 | 90.3 | 22.1 | 22.2 | 1.4 | 11.3 |
| LLE Cartridge ISTD | 73.4 | 48.9 | 54.8 | 57.8 | 129.3 | 57.1 |
| LLE hand ISTD | 644.4 | 4.6 | 16.9 | 28.8 | 30.2 | 104.3 |
| Freeze Dry DBP | 30.0 | 131.9 | 89.6 | 68.0 | 88.0 | 108.2 |
| LLE Cartridge DBP | 114.4 | 166.1 | 154.0 | 119.5 | 82.5 | 62.4 |
| LLE hand DBP | 56.1 | 817.2 | 1184.0 | 2750.8 | 3087.7 | 3670.8 |







## PRECISION OF THREE CLEAN UP METHODS WITH GC -MS/MS




## CONCLUSION

Dialkylphosphates (DAPs) were measured using three different clean-up methods (freeze dry, hand LLE, cartridge LLE) with two different instruments (GC-MS/MS, GC-MSD) and two internal standards (label internal standard, surrogate dibutylphosphate standard). Overall in terms of analyte recoveries, detection limits, precision and accuracy, the freeze dry clean-up method with analysis on the GC-MS/MS using label internal standard is superior. Investigation of analyte recoveries and detection limits of several other combination methods, although inferior to the freeze dry/GC-MS/MS/label internal standard method, is sufficient for an analytical method to determine high level concentrations of DAPs expected to be found in chemical terrorism situations. Conversely, the loss of precision and accuracy is much more significant when looking at less expensive methods of analysis. The selected cost-effective method for chemical terrorism situations is the cartridge clean-up with label internal standard (as the surrogate internal standard drastically decreases precision and accuracy) with GC-MSD. Although recoveries were low (25-85%), detection limits range from 48-129 ng/mL which is suitable for chemical terrorism events. In addition, all analytes were highly linear with correlation coefficients between 0.97 – 0.99.

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